ACTIVITIES OF SOME CARBOHYDRATE METABOLISING ENZYMES IN BOTH THE YEAST-LIKE AND MYCELIAL-LIKE FORMS OF *CANDIDA ALBICANS*

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INTRODUCTION

Most of the work on enzymes in *Candida albicans* so far deals mainly with the yeast form and little is known about the mycelial form. This organism readily infects diabetic patients, in whose system the carbohydrate metabolism is altered. Susceptibility to this pathogen is also enhanced during pregnancy which is accompanied by a considerable increase in the glycogen content of the vagina (Skinner, 1947). Tissue invasion by this fungus is associated with the appearance of mycelia (Young, 1958). Since this suggests that carbohydrate may play a part in the yeast-mycelia change, a study of some enzymes involved in carbohydrate metabolism in both forms may throw some light on the morphogenesis of this fungus. The enzymes selected were those involved in the three main cycles of glucose dissimilation, namely the glycolytic and tricarboxylic acid cycles and the hexose monophosphate pathway.

MATERIALS AND METHODS

The strain of *Candida albicans* used for the enzyme studies is from the vaginal region of a pregnant patient. Growth of both the yeast and mycelial forms of the organism was as described (Ton and Karunairatnam, 1976). The cells were spun down at 0-2°C in a Sorvall centrifuge and the harvested cells (both yeast and mycelia) were used for the enzyme assays.

The harvested yeast or mycelial cells were mixed with twice their weight of alumina 305. The cells were ground in a precooled mortar for 10-15 min at 0-2°C. The enzymes were extracted with 0.03M phosphate buffer, pH7.0 and the extract centrifuged at 14,000g_{av} for 30min in a Sorvall centrifuge at 0-2°C. The crude cell-free supernatant was dialyzed against 0.003M phosphate buffer, pH7.0 at 0-2°C for 20h. The dialysed extract was used for the enzyme study.

Galactokinase and hexokinase were determined by the method of Leloir and Trucco (1955) while the method employed by Slein (1955) was used for the estimation of phosphoglucose isomerase. Aldolase was measured by the colorimetric method of Sibley and Lehninger (1949). Pentose phosphate isomerase was estimated according to the procedure of Axelrod and Jang (1954). The enzymes glucose-6-phosphate and 6-phosphogluconic dehydrogenases were measured by following the reduction of NADP at 340nm (Demoss, 1955). The presence of citrate condensing enzyme was detected by the method of Ochoa (1955) while aconitase was assayed by measuring the increase in extinction values at 240nm with citrate (Racker, 1950).

The method employed by Massey (1955) was used for detecting fumarase and isocitric dehydrogenase was measured by the reduction of NADP at 340nm. The reduction of ferricyanide at 400nm was used for estimating succinic and α-ketoglutaric dehydrogenases (Slater and Bonner, 1952).

The protein content of the dialysed cell extract was determined according to Lowry *et al.*, (1951) using bovine serum albumin as standard. DNA from the cells were extracted by the procedure of Smellie and Krotkov (1960) and the extract was assayed by the diphenylamine reaction (Burton, 1956). The statistical analysis student's 't' test was used as set out in Croxton *et al.*, (1969). The confidence level was taken to be 95%.

RESULTS

Enzyme studies on crude cell-free extracts indicated the presence of enzyme of the glycolytic pathway, hexose monophosphate shunt and the tricarboxylic acid cycle in both forms of the organism (Table 1). No significant difference was detected in the specific activities of the enzymes as well. However, the presence of galactokinase was detected only in the mycelial cells (Table 2). It has been found that galactokinase could be induced in yeast grown in the presence of galactose. In this strain of *Candida albicans*, besides the induction of the enzyme, there was a change in the morphology of the fungus, namely a yeast mycelia transformation has occurred. Protein to DNA ratio for both forms is shown in Table 3. The value for the mycelia form was about 1.5 times higher than that for the yeast form. This difference could be due to the synthesis of new proteins in the mycelial form.

DISCUSSION

Generally if the yeast to mycelia transformation involves simple elongation followed by nuclear division, the protein to DNA ratio of both forms should be equal. If, however, the mycelium is formed by simple elongation

Table 1

Some enzymes in the glycolytic cycle, hexose monophosphate shunt and the citric acid cycle in crude cell-free extract of the yeast and mycelial forms of *Candida albicans*.
*= standard deviation; figure in parentheses denotes the number of assays done;
(√) = enzyme assay carried out but not quantitated.

Enzyme assayed	Specific activities (enzyme units/mg protien)	
	Yeast form	Mycelial form
Phosphoglucose isomerase	475 ± 22.0(4)	516 ± 61.8(4)
Aldolase	11.5 ± 1.5(7)	18.3 ± 1.9(5)
Glucose-6-phosphate dehydrogenase	0.78 ± 0.11(7)	1.25 ± 0.16(9)
6-phosphogluconic dehydrogenase	0.42 ± 0.05(6)	0.5 ± 0.05(6)
Pentose phosphate isomerase	7.6 ± 1.05(5)	11.5 ± 2.5 (6)
Fumarase	0.36 ± 0.17(4)	0.32 ± 0.04(4)
Isocitric dehydrogenase	420 ± 40.3(5)	459 ± 123(5)
Succinic dehydrogenase	5.4 ± 0.05(5)	2.7 ± 0.42(5)
α-ketoglutaric dehydrogenase	√	√
Hexokinase	√	√
Citrate condensing enzyme	√	√
Aconitase	√	√

Table 2

Galactokinase activity in crude cell-free extract of the yeast and mycelial forms * = standard deviation; figure in parentheses denotes the number of assays done.

Form	Specific activities (enzyme unit/mg protein)
Yeast	0.0 (7)
Mycelia	2.2 ± 0.18* (6)

Table 3

Protein/DNA ratio in crude cell-free extract of yeast and mycelial forms * = standard deviation; figure in parentheses denotes the number of assays done.

Form	Protein/DNA ratio (expressed as μg protein/μg DNA)
Yeast	56.4 ± 5.6*(10)
Mycelia	76.4 ± 4.4(5)

without nuclear division this would be different from that in the yeast. Results in Table 3 indicated that the protein to DNA ratio in the mycelial form was almost 1.5 times that in the yeast form. This suggests that the changes involved maybe such that elongation occurred more rapidly than nuclear division. This elongation appears to be useful for the fungus, particularly in utilising food material, which may be concentrated in a zone remote from its point of entry, throughout its entire length. It may also be playing a part in the invasion and colonisation of tissue because it is easier for this form to spread as compared with the ovoid form.

What is striking in the above study is that in spite of the morphological difference in the yeast and mycelial forms of the organism, there was no significant difference in the enzyme activities of the glycolytic pathway, the tricarboxylic acid cycle and the hexose monophosphate shunt. The fact that there was not much difference in the enzyme activities could support the postulation that elongated cells of micro-organisms may be viewed as a result of differential inhibition of cell division without concomitant inhibition of metabolic processes necessary for the growth of the cells.

It has been found that comparative studies of the metabolism of a normal and a division-less mutant strain of *C. albicans* led to demonstrations of equivalence in growth rates, nutritional requirements, respiratory activity, polysaccharide composition and levels of cystine and glutathione reductase (Romano and Nickerson, 1954; Ward and Nickerson, 1958). However, some qualitative changes in electrophoretic bands of water soluble proteins of the budding and filament forming cells of *C. albicans* have been reported by Dabrowa *et al.*, (1970). Changes in the specific activities of DNA-dependent RNA polymerases in crude extracts of the dimorphic fungus *Mucor rouxii* during the transition from the yeast to the mycelial forms were observed (Young and Whiteley, 1975). Work is now underway to determine whether DNA metabolising enzymes may be involved in such transition though our data suggest that this transition may be related to or is dependent upon carbohydrate metabolism.

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